

Oyster Shell as pH Control Substitute for Two Stage Anaerobic Co-Digestion System

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Alkali substitute for two-stage anaerobic co-digestion system using kitchen waste, agriculture waste, and horse dung was compared with the standard alkali chemical used for pH control. The substitute used in this experiment is oyster shell waste. The study aims to investigate and evaluate the performance of oyster shells to overcome problems such as pH drops in the hydrolysis stage, greenhouse gas emission from chemical use, and methanogenesis stage performance such as methane gas yield and quality of biogas production. The result shows hydrolysate from control treatment using alkali has the highest volatile solid (VS) (1.18 %) and chemical oxygen demand (COD) (27.7 g/L). Oyster shell treatment has lower VS (1.14 %) and COD (25.01 g/L), but not significantly different from control. One-time oyster shell application can maintain reactor pH throughout the acid phase. Alkali treatment needs multiple doses to maintain the reactor in the intended pH range. At the end of the first stage, liquid hydrolysate pH value from alkali, oyster shell and no pH control treatment are 6.5, 5.29 and 3.8. As a result of comparing greenhouse gases emission in the cases of using NaOH as alkali, and oyster shell by Life Cycle Assessment (LCA) method, equivalent weight of CO₂ emission from the oyster shell is lower by 0.0504 kg-CO₂/kg-waste than that from alkali or only 0.15 times. In methanogenesis stage, alkali treatment produced 31 % higher methane gas yield (429.82 mL CH₄/g VS in 1.64 g VS/L/d) compared to oyster shell treatment (321.32 mL CH₄/g VS in 2.8 g VS/L/d). Methane gas yields of oyster treatment are stable through the different range of organic loading rate (OLR) while in alkali treatment the gas yield tends to increase in proportion with higher OLR. Highest methane gas percentage in biogas produced from alkali treatment (77.53 %), while in oyster shell treatment highest methane gas percentage is 71.11 %. Alkali treatment performs better in methane gas production using two stage anaerobic digesters, but oyster shells offer advantages in terms of practicality, stability and environmental impact

1. Introduction

The global waste issue is becoming worse as the volume of waste increases from the rising population and living standard in developing countries. There are various types of anaerobic digestion (AD) systems that able to convert organic waste into energy by utilizing microbiological processes. Food waste, livestock waste, and agricultural waste are common feedstock for AD due to biodegradable materials' high composition. However, AD processes with mono-substrate usually showed instability, and, in this case, remedial measures were introduced by mixing different feedstock or co-digestion (Kim et al., 2011). Co-digestion also allows AD system to have higher biochemical methane potential (Ivana et al., 2016) and achieve better nutrient balance (Bong et al., 2018) than mono-digestion system.

The two-stage methane gas generation system executes the former from hydrolysis to acidogenesis and the latter process from acidogenesis to methanogenesis in different reactors separately. The advantages of using a two-stage anaerobic system compared to the conventional single-stage system include, but not limited to, higher conversion of carbon into biogas, faster methanogenesis kinetics, and higher CH₄ content (Dinh et al., 2019). Nevertheless, this system still experiences an issue, namely pH drops in hydrolysis and acidogenesis process. pH drops in the acidogenesis process originated from the conversion of carbohydrates into volatile fatty acids (VFA). The alkali commonly used is NaOH or KOH to reduce the acidity quickly. However, such

expensive chemical leads to high operational cost and generates more CO₂ emissions from alkali chemical manufacture.

The oyster shells which contain CaCO₃ at 80-95 % (de Alvarenga et al., 2012) has the potential to be able to control the pH instead of NaOH or KOH. Some oyster shells are used as fertilizer, soil conditioner, or water treatment, but for the most part, they are piled up on seashore as fishery waste material (Oo et al., 2018). In 2017 total oyster production from capture are 148 t and 5,711 t from aquaculture (FAO, 2019). Data on the amount of oyster shell waste was not recorded, and the weight percentage of shells also varied according to the species. A study by Tokeshi et al. (2000) stated that shell weight could range from 58.6 % to 76.4 %. This re-use of oyster shells might help for the reduction of waste and waste treatment.

CaCO₃ itself has been used by Chen et al. (2015) in a single-stage batch AD system of food waste and had a remarkable effect on stabilization. This stabilization property is quite essential to prevent system failure or change in OLR for an extended period. However, few studies have been conducted to investigate the effect of alkali source that mainly consist of CO₃²⁻ in 2 phase system. This study examines and evaluates the performance of oyster shells as alkali material in controlling the pH of the two-stage anaerobic system.

2. Materials and methods

2.1 Material

As raw materials for AD, kitchen waste (KW), agriculture waste (AW) and horse dung (HD) were collected from a campus cafeteria, an agriculture faculty field, and a stable in Tsushima Campus, Okayama University. After chopping the raw materials by food cutter (Mizinger, Chiba Kogyo Co., Ltd.), drying in drying oven at 105 °C (DV340S, Yamato Science Co., Ltd.) and burning in muffle furnace at 550 °C (FO300, Yamato Science Co., Ltd.), total solid (TS) and volatile solid (VS) for each material were analysed. The results are shown in Table 1.

Table 1: Material properties

Material	TS (%)	VS (%)
KW	46.40	44.43
AW	13.77	11.85
HD	22.14	20.13

2.2 Anaerobic digestion system

Figure 1a shows configuration of the two-stage anaerobic. First stage is hydrolysis reactor, consisted of continuous stirred tank reactors (CSTR) (2 L, TS 3 %, HRT 5 d, 35°C) operated in batch mode and replicated three times for all treatments. Second stage is using up-flow anaerobic sludge blanket reactor UASBR (6 L, 35 °C) as methane reactor. The bacterial cultures for methanogenesis and hydrolysis reactor came from horse dung. Experimental material was prepared by mixing KW, AW, and HD in the TS % ratio of 6:3:2. When pH dropped lower than initial pH value, crushed particle of oyster shells was put into the hydrolysis reactor at a ratio of 7.5 g per material volume 1 L. On the other hand, as control runs, one treatment group used NaOH 10M for pH control and another treatment group pH was not controlled. Hydrolysate diluted with water with various ratio (feedstock: water) before pumped into the reactor. The dilution ranges from 1:2, 1:1, 2:1, and 5:1. It also affect HRT that range from 3 d to 10 d. Table 2 listed the operation parameter and this variation has been set to keep pump operated as close as 24 h.

Table 2: Operation of methane reactor

Alkali Treatment			Oyster Shell Treatment			No pH Control Treatment		
OLR (g VS/L/d)	Dilution	HRT (d)	OLR (g VS/L/d)	Dilution	HRT (d)	OLR (g VS/L/d)	Dilution	HRT (d)
0.4	1:2	10	0.4	1:2	10	0.4	1:2	10
0.8	1:2	5	0.8	1:1	5	0.8	1:2	5
1.2	1:1	5	1.2	1:1	5	1.2	1:1	5
1.4	2:1	5	1.6	1:1	4	1.6	5:1	5
1.6	2:1	5	2.0	1:1	3	2.2	5:1	4
2.0	2:1	4	2.4	5:1	3			
2.4	5:1	4	2.8	5:1	3			
2.6	5:1	4						

At the solid-liquid separation after hydrolysis reactor, samples of solid and liquid hydrolysate were analysed for TS and VS characteristic as well as COD parameter by using spectrophotometer (MD600, Lovibond). CH₄ and CO₂ percentages of biogas produced in methanogenesis reactor was analysed with gas chromatography (GC-2014, Shimadzu, Japan).

In environmental impact evaluation of alkali case, the weight of NaOH consumed in the experiment was accounted and GHG emission inventory of NaOH production was estimated by using SimaPro 9.0.0 software. The system boundary for NaOH can be seen in figure 1b within square a. Result of assembly of NaOH (materials, production, transport, and energy processes that needed for production) was then compared with oyster shell. In the oyster shells case, GHG emission inventory of production of the oyster shells powder was estimated based on study of Boicko (2005) in Alvarenga et al. (2012) and the system boundary is within square b of figure 1b. The powder is produced from waste of oyster shells with milling machine and dryer. The electric power required by the machines was estimated and was referenced in calculation of GHG emission. The GHG emission from treatment of the undissolved powders was not considered because undissolved oyster shells powder ends up to composted solid residue. Also, GHG emission in terms of dilution with water, heating and stirring for reactors, and water treatment of the effluent from methanogenesis reactor, and so on was not considered because such GHG emission is same as the oyster shell case.

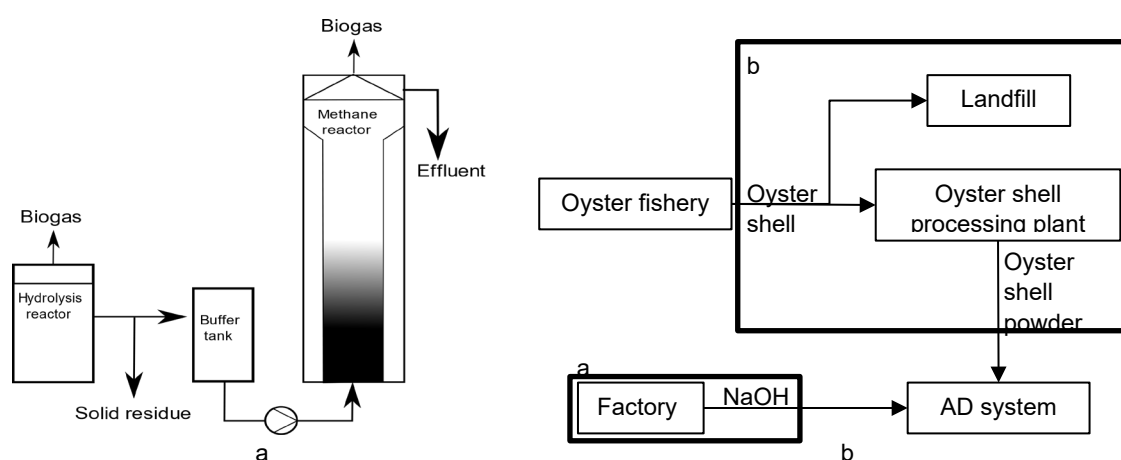


Figure 1: Configuration of (a) two-stage AD system and (b) system boundary of GHG emission calculation

3. Results and discussion

3.1 TS, VS and COD of hydrolysate

In the process of the hydrolysis reactor, the organic composition of the raw material changes into simpler and soluble form by hydrolytic and acidogenic bacteria, leaving the inorganic fraction of ash. After five days from the beginning of experiment, TS, VS and COD level of the hydrolysate were analysed. The results are shown in figure 2.

In the experiment using oyster shell powder for pH control, ash content in the solid residue was higher than other experiment conditions because undissolved oyster shells remained. On the other side, in the case of alkali input, the amount of ash in the liquid hydrolysate is high. It might be the result of salt formation from acid and alkali (Bethi et al., 2020). The ash content itself represents inorganic fraction of TS.

In the case of no pH control, the amount of VS in the solid residue was the highest, but VS in the liquid residue was the lowest. In this instance, the hydrolysis process, in which microorganism digested the complex organic matter of solid raw material and transferring it as soluble component in the liquid residue, was inhibited. There are still many organic components in the solid part, not yet hydrolysed. The hydrolysis process reduced only 57.16 % of VS from raw material. It is lower than alkali treatment that can reduce 73.33 % of VS from material and oyster shell treatment with 67.03 % reduction of VS as well. Because of that alkali treatment have the highest amount of VS in liquid residue with VS 1.18 %, followed by reactor with oyster shell pH control with VS 1.14 % and no pH control with VS 1.12 %.

For better understanding of hydrolysis result, COD_{Cr} value also measured to analyse the amount of organic substance in liquid residue. Looking from COD_{Cr} of liquid residue in figure 2b, the result did not differ from VS measurement. Liquid residue from hydrolysis with alkali had the highest COD_{Cr} (27.97 g/L), followed by the case with oyster shells (23.88 g/L) and that without pH control (20.83 g/L). The case with alkali had the lowest

amount of VS in the solid residue, and the highest VS in liquid residue, that is, VS reduction from the solid part in the hydrolysis phase is the highest. Consequently, the method to input alkali for pH control resulted in the best to enlarge organic matter in liquid phase in hydrolysis process.

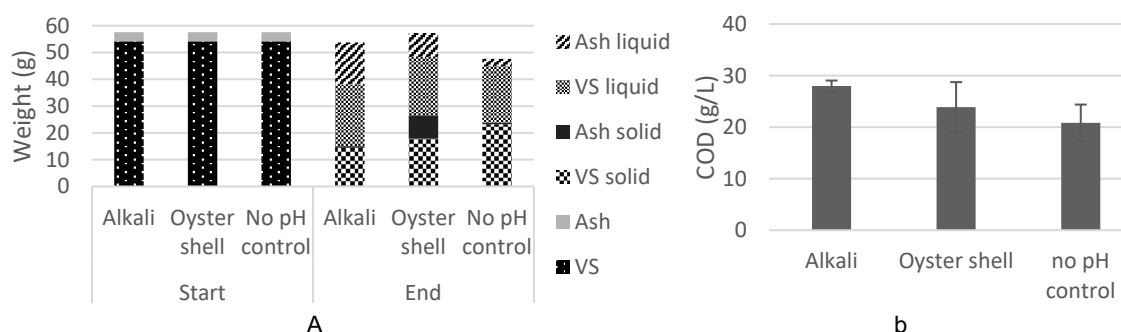


Figure 2: (a) TS composition and (b) COD_{cr} of hydrolysate from acid fermentation phase

3.2 pH in acid fermentation phase

Hydrolysis and acidogenesis in first phase of this system produced Volatile Fatty Acid (VFA) that reduced pH value of the reactor. As seen in Figure 3, without pH control the pH quickly dropped to 3.97 in one day. Hydrolytic microbes can grow fast and endure against its environmental variation such as temperature and pH changes in some degree. However, pH below 4 are outside of its optimum pH range around 6 - 7 (Trzcinski and David., 2017). The general optimum pH range of acidogenic microbes are 6 - 6.5 (Ren et al., 1997) and when the pH is lower than 5.5 it will produce more ethanol than VFA (Li et al., 2010). This pH condition produced high VS and COD_{cr} of liquid residue in hydrolysis reactor. In the case of oyster shells powder addition of the powder raised the pH value at the start of experiment and maintained at around 5.5 until stopping at 5.3 on the fifth day. As the pH value could not exceed 6, the results of VS and COD_{cr} were below those of alkali case.

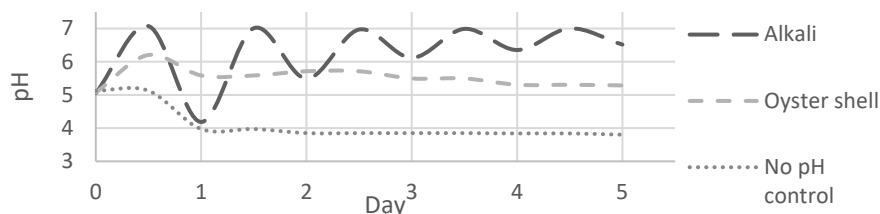


Figure 3: pH value of acid fermentation phase

Using pure chemical CaCO₃ may resulted in better pH control as seen in study by Chen et al. (2015). Based on that study, the pH of AD using CaCO₃ only reduced to slightly below 6.5 on the second day. On the other hand, using waste eggshell consisted of 94.52 % CaCO₃ dropped the pH at the lowest point of 5.25 - 5.5 on third day. These conditions affected the methane production and the start of methanogenesis process. The effect of pH on hydrolysate also observed on hydrolysis of two-stage AD system using kitchen waste (Zhang et al., 2005). The solubilization based on COD content observed higher in pH 7 than pH 5. However higher pH than 7 does not result in better solubilization.

3.3 Methane gas production

Methane gas yield and percentage were measured after feeding the liquid residue to the methanogenesis reactor with different hydrolysate concentrations. First of all, the methane yield ratio is shown in figure 4a. In the alkali case, the methane yield ratio rose from 240 to 320 (mL/g VS) according to the increase of the organic loading rate (OLR). In the oyster shells case, the yield ratio was stable at around 320 mL/g VS. The former yield ratio was lower than the latter yield at lower OLR (~0.8 g VS/L/d) but was vice versa at higher OLR (1.5 g VS/L/d~). In the highest OLR, the methane yield of alkali treatment and oyster shells is 422 mL/g VS and 321 mL/g VS. However, a higher VS reduction percentage in the effluent of the highest OLR detected in oyster shell treatment with 63 % VS reduction than in alkali with only 38 % VS reduction. This observation of reactor removal

efficiency needs further examination in longer time range. According to Dinh and Luan (2020), the removal efficiency of reactor will gradually improve over time.

In the next graph, methane and CO₂ percentages are shown in figure 4b. In the alkali case, CH₄ percentage was quite higher than the case without pH control, and it had a peak of 77.53 % at 2.0 (g VS/L/d). CO₂ percentage was stable under 25 %. In the oyster shells case, CH₄ percentage was around 70 % (66.55 - 71.11 %) at the range from 0.8 to 2.8 (g VS/L/d). Since methane percentage of methanogenesis biogas is generally around 50 – 75 % and CO₂ percentage are about 25 – 50 % (Roubik et al., 2018), it was identified that methane percentage of this two-stage methane fermentation system with pH control was relatively high.

Finally, in the case without pH control, the methane yield ratio was quite lower, around 200 (CH₄/g VS) than other cases. The methane percentage versus OLR has a decreasing trend that is quite different from other cases. Trends of CO₂ percentage of all cases were contrary to those of methane percentage. Generally, mildly acidic pH inhibits methanogen (Ye et al., 2012) to converts acetate by acetoclastic methanogen, or H₂ and CO₂ into methane by hydrogenotrophic methanogen (HM). In addition to this, higher OLR shifts the pathway to the syntrophic acetate oxidizing (SAO) pathway (Bi et al., 2020) that convert acetate to H₂ and CO₂ before HM converts it to methane. This combination of methanogen inhibition and pathway will shift further lower methane production and leave the intermediate product of the SAO pathway, which one of them is CO₂, to accumulate. In the case without pH control treatment with the lowest pH, this inhibition and intermediate product accumulation may affect the biogas production in the form of low methane content and higher CO₂ content. A slight increase in CO₂ percentage in the oyster shell case may be generated from the same way, by the methanogen inhibition due to acidic pH (5.3 pH value) and CO₂ accumulation from the SAO pathway.

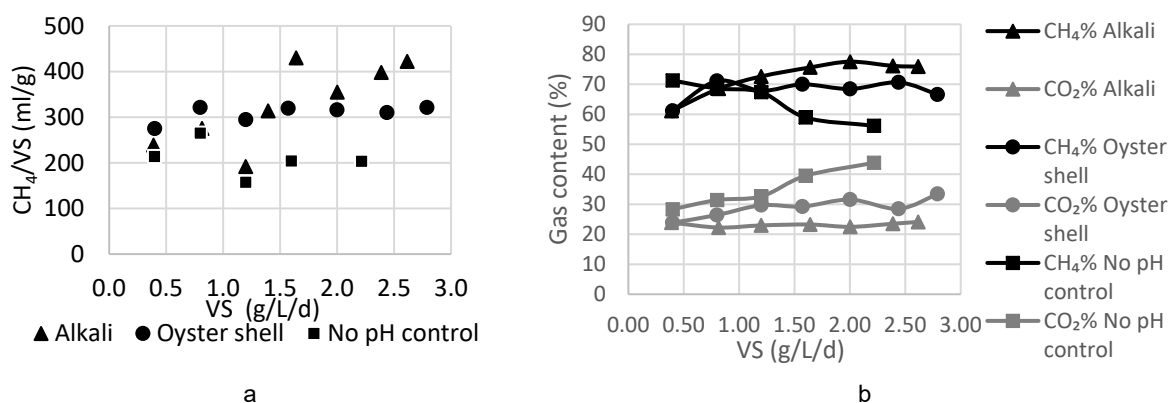


Figure 4: Methane yield (a) and gas content (b)

3.4 Evaluation in greenhouse gas emission

The result of GHG emission in the two cases is shown in Table 3.

Table 3: GHG emission calculation

Material	pH conditioner per experimental material (kg/kg)	GHG emission per pH conditioner (kg CO ₂ -eq/kg)	GHG emission per experimental material (kg CO ₂ -eq/kg)
Alkali (NaOH)	0.0463	1.26	0.0583
Oyster shell powder (CaCO ₃)	0.0864	0.102	0.0088

The pH conditioner use per unit waste in the alkali case was lower than that in the oyster shells case. It can be considered a good point because the more oyster shell can be used, the more oyster shell waste amount reduced. GHG emission rate of NaOH is much bigger than that of the oyster shells case, so that resulting in GHG emission of the oyster shells case at 0.15 times of the alkali case. This result indicates a large GHG reduction is expected if oyster shell powder is used as pH conditioner in two-stage methane fermentation.

4. Conclusions

Using the oyster shell in an anaerobic digester can prevent the acid fermentation phase from going too acidic with only one-time application compared to alkali that needs constant monitoring and application. Oyster shells

application can maintain pH from 5.5 to 5.3 at the end of hydrolysis. The methane yield in oyster shell case in higher OLR (2 – 2.8 g VS/L/d) is lower than using hydrolysate with alkali treatment. Highest methane yield in oyster shell treatment, 321.32 mL CH₄/g VS (OLR 2.8 g VS/L/d), is lower than the highest methane yield in alkali treatment, which is 429.82 mL CH₄/gVS (OLR 1.64 g VS/L/d). However, the yield is stable, whether in low or higher OLR, fluctuating in around 320 mL CH₄/g VS. This stability is an excellent point to have because the amount of waste is not consistent. Using oyster shells as a substitute is more environmentally friendly, based on the reduction of GHG emissions up to 0.15 times than from using alkali. Based on this study, oyster shells can be used as an alkali substitute in controlling pH of two-stage AD with some advantages. Longer period of oyster shell powder usage as substitute still need to be investigated for the next study, as well as microbiological analysis for further understanding of the process in the system.

References

- de Alvarenga R.A.F., Galindro B.M., de Fátima Helpa C., Soares S.R., 2012, The recycling of oyster shells: An environmental analysis using Life Cycle Assessment, *Journal of Environmental Management*, 106, 102-109.
- Bethi C.M., Narayan B., Martin A., Kudre T.G., 2020, Recovery, physicochemical and functional characteristics of proteins from different meat processing wastewater streams, *Environmental Science and Pollution Research*, 1-13.
- Boicko A.L., 2005, Use of *Crassostrea gigas* oysters as filler for polyvinyl chloride (PVC) products. Course Conclusion Paper in Materials Engineering, Federal University of Santa Catarina (UFSC), Florianopolis, Brazil (In Portuguese).
- Bong C.P.C., Lim L.Y., Lee C.T., Klemeš J.J., Ho C.S., Ho W.S., 2018, The characterisation and treatment of food waste for improvement of biogas production during anaerobic digestion—A review, *Journal of cleaner production*, 172, 1545-1558.
- Chen S., Zhang J., Wang X., 2015, Effects of alkalinity sources on the stability of anaerobic digestion from food waste, *Waste Management & Research*, 33(11), 1033-1040.
- Dinh N.T., Luan T.N., 2020, Study on the Performance of Internal Circulation (IC) Anaerobic Digester Treating High Strength of Food Processing Wastewater: Effect of Organic Loading Rate, *Chemical Engineering Transactions*, 78, 505-510.
- Dinh P.V., Takeshi F., Minh G.H., Phu S.T.P., 2019, Comparison Between Single and Two-Stage Anaerobic Digestion of Vegetable Waste: Kinetics of Methanogenesis and Carbon Flow, *Waste and Biomass Valorization*, 1-9.
- Environmental Protection Agency, 2020, Greenhouse gas equivalencies calculator <www.epa.gov/energy/greenhouse-gas-equivalencies-calculator> accessed 29.05.2020
- FAO, 2019, Fishery and Aquaculture Statistics 2017, FAO yearbook, Rome, Italy.
- Ivana C., Maríaa T., Auraa V., Paolaa A., Marioc H., 2016, Anaerobic co-digestion of organic residues from different productive sectors in colombia: Biomethanation potential assessment, *Chemical Engineering Transactions*, 49, 385-390.
- Kim D.H., Kim S.H., Kim H.W., Kim M.S., Shin H.S., 2011, Sewage sludge addition to food waste synergistically enhances hydrogen fermentation performance, *Bioresource Technology*, 102(18), 8501-8506.
- Meegoda J.N., Li B., Patel K., Wang L.B., 2018, A review of the processes, parameters, and optimization of anaerobic digestion, *International Journal of Environmental Research and Public Health*, 15(10), 2224.
- Oo A.Z., Sudo S., Win K.T., Shibata A., Gonai T., 2018, Influence of pruning waste biochar and oyster shell on N₂O and CO₂ emissions from Japanese pear orchard soil, *Heliyon*, 4(3), e00568.
- Ren N., Wang B., Huang J.C., 1997, Ethanol-type fermentation from carbohydrate in high rate acidogenic reactor, *Biotechnology and Bioengineering*, 54(5), 428-433.
- Roubík H., Mazancová J., Le Dinh P., Dinh Van D., Banout J., 2018, Biogas quality across small-scale biogas plants: A case of central Vietnam, *Energies*, 11(7), 1794.
- Trzcinski A.P., David C.S., 2017, Microbial biomethane from solid wastes: Principles and biotechnological processes, Chapter In: F.D. Harzevili, I.S. Hiligsmann (Eds.), *Microbial fuels: technologies and applications*, CRC Press, Boca Raton, USA, 77-168.
- Ye R, Jin Q, Bohannan B, Keller J.K, McAllister S.A, Bridgham S.D, 2012, pH controls over anaerobic carbon mineralization, the efficiency of methane production, and methanogenic pathways in peatlands across an ombrotrophic–minerotrophic gradient, *Soil Biology and Biochemistry*, 54, 36-47.
- Zhang W., Dai K., Xia X.Y., Wang H.J., Chen Y., Lu Y.Z., Zhang F., Zeng R.J., 2018, Free acetic acid as the key factor for the inhibition of hydrogenotrophic methanogenesis in mesophilic mixed culture fermentation, *Bioresource Technology*, 264, 17-23.